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Estimation of intradermal disposition kinetics of drugs: II. Factors determining penetration of drugs from viable skin to muscular layer

Kazutaka Higaki, Masahide Asai, Takayuki Suyama, Kazuki Nakayama, Ken-ichi Ogawara, Toshikiro Kimura *

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan

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Abstract

To develop a more efficient transdermal delivery system, it is very important to regulate the intradermal disposition of drugs after topical application. We tried to elucidate the factors determining the intradermal disposition kinetics, especially drug penetration from the viable skin to the muscular layer, mainly based on the six-compartment model, including the contralateral skin and muscle for ten model drugs with different physicochemical characteristics. In vivo transdermal absorption study was performed for six model drugs using the stripped-skin rats. The fitting analyses by the six-compartment model gave the theoretical curves describing the observed data very well and the reasonable pharmacokinetic parameters, showing the pharmacokinetic model should be useful for the estimation of the intradermal disposition kinetics of drugs applied topically again. The simulation study using the pharmacokinetic parameters obtained above could show the relative contribution of the direct penetration and the distribution from the systemic circulation to the muscular distribution of drugs. The largest contribution of direct penetration was observed for antipyrine (90.8%) and the smallest was for felbinac (43.3%). Among the pharmacokinetic parameters obtained above, the clearance from the viable skin to the muscle (CL_{vs-m}) was found to be significantly correlated with the unbound fraction of drugs in the viable skin (fu_{vs}). Although the clearance from the viable skin to the plasma (CL_{vs-p}) also tended to increase as fu_{vs} increased, the ratio of CL_{vs-m} to CL_{vs-p} was significantly correlated with fu_{vs}, meaning that the larger amount of unbound drug in the viable skin significantly contributes to the direct penetration into the muscle more than to the systemic absorption. On the other hand, k_{direct} values obtained in in vitro penetration study—the penetration rate constant of drugs from the viable skin to the muscular layer—were found to be correlated with CL_{vs-m} values for seven model drugs. Therefore, adding the in vitro experiments for the other three model drugs, the multiple linear regression analysis of k_{direct} was performed for ten model drugs in terms of fu_{vs}, logarithm of the partition coefficient (Log P) and molecular weight. The results clearly showed the largest and significant contribution of fu_{vs} to the direct penetration of drugs from the viable skin to the muscular layer, indicating

* Corresponding author. Tel.: +81-86-251-7948; fax: +81-86-251-7926.
E-mail address: kimura@pheasant.pharm.okayama-u.ac.jp (T. Kimura).

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that a drug with the higher value of fu_{vs} in the viable skin can penetrate more into the muscular layer.

Keywords: Intradermal disposition; Unbound fraction; Multiregression analysis; Penetration into muscular layer; Systemic absorption; Distribution from systemic circulation

1. Introduction

It is very important in the development of a more efficient transdermal delivery system to regulate the intradermal disposition of drugs after topical application because some drugs are expected to be absorbed systemically and others are to be distributed to the local tissue, such as a muscle. Since the stratum corneum permeability to drugs has recently been enhanced by several approaches (Hashida et al., 1985; Hadgraft et al., 1985; Williams and Barry, 1992; Singh et al., 1995; Nakamura et al., 1996; Palagiano et al., 1997; Riviere and Heit, 1997), the regulation of drug disposition kinetics in the dermal tissues must be the next objective for the development of the transdermal delivery system. We have already developed the six-compartment model, including the contralateral tissues, which described the intradermal disposition of antipyrine after topical application very well (Nakayama et al., 1999). The analysis based on the model showed that antipyrine distributed to the muscle was mainly attributed to the direct penetration from the viable skin. However, how drugs applied topically can reach the deeper tissue, i.e. the muscular layer, is still controversial. Estradiol, progesterone (Marty et al., 1989), salicylate (Singh and Roberts, 1993; Cross et al., 1997) and piroxicam (M-Riviere et al., 1993) were reported to penetrate into the local deep tissue after topical application, but Radermacher et al. (1991) presented that diclofenac was distributed to the underlying deep tissue mainly via systemic circulation after topical application. Furthermore, it was also reported that biphenylacetic acid in the deeper tissue was mainly derived from the systemic blood supply after being applied topically (Dawson et al., 1988).

In the present study, ten model drugs, including antipyrine (Nakayama et al., 1999), were examined in terms of the intradermal disposition kinetics based on the six-compartment model, using the stripped-skin rats. Then, we tried to estimate how the direct penetration contributed to the drug disposition in the muscular layer and to elucidate factors determining the drug penetration from the viable skin to the muscular layer.

2. Materials and methods

2.1. Materials

Diclofenac sodium, ketoprofen, felbinac, flurbiprofen, naproxen sodium, tolmetin sodium and zomepirac sodium were purchased from Sigma Chemical Co. (St. Louis, MO). Salicylic acid, propranolol hydrochloride and antipyrine were obtained from Ishizu Pharmaceutical Co. (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), Tokyo Chemical Industry Co. (Tokyo, Japan), respectively. All other reagents were of the highest grade commercially available.

2.2. Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan), maintained at 25 °C and 55% humidity, were allowed free access to standard laboratory chow (Clea Japan, Tokyo) and water prior to the experiments. Rats weighing 230–270 g were randomly assigned to each experimental group. Our investigations were performed after approval by our local ethical committee at Okayama University and in accordance with 'Interdisciplinary Principles and Guidelines of the Use of Animals in Research'.

2.3. In vivo transdermal absorption study

Abdominal hair was removed using 7% thioglycolic acid gel 2 days before performing the absorption study and the stratum corneum was

stripped with adhesive tape about 20 times under urethane anesthesia just before starting the absorption study. Under urethane anesthesia, 2 ml of a model drug solution (5 mM) was applied to the donor cell, of which effective area was 4.91 cm^2, attached on the stripped abdominal skin with Aron Alpha (Toa Chemicals Co. Ltd., Tokyo). After the rats were sacrificed at 0.5, 1, 2, 3, 4 and 7 h, concentrations of the drug in the donor cell, viable skin, muscle, contralateral skin, contralateral muscle and plasma were determined.

2.4. In vitro penetration study

According to the procedure of in vivo transdermal absorption study, a model drug solution in the donor cell was applied onto the stripped skin for 2 h. Then, the donor cell was removed and the stripped skin was carefully excised with the muscular layer below the skin. The isolated skin-muscle sheet was mounted in a Franz-type diffusion cell. The area for diffusion was 3.14 cm^2 and the receptor compartment was filled with 18 ml of an isotonic phosphate buffer (pH 7.4). The diffusion cell was thermoregulated with a water jacket at 37 °C and the receptor compartment was stirred with a magnetic stirrer. At fixed time periods to 6 h, the concentration of drugs in the viable skin was determined and the penetration rate constant from the viable skin to the muscular layer (k_{direct}) was calculated.

2.5. Determination of unbound fraction in viable skin homogenate

A stripped skin was homogenized with twice the volume of ice-cold buffer (pH 7.4) and the homogenate was filtered through gauze to remove the fibers. Finally, the 27% (w/v) homogenate containing a model drug at 1 mM was obtained. After incubation at 37 °C for 10 min, an aliquot of the incubation mixture was applied to a Centrifree Micropartition System (Amicon Inc., Beverly, MA) and the ultrafiltration was performed at $1300 \times g$ for 10–20 min. The drug concentration in the filtrate was determined and the unbound fraction, fu_{vs}, was calculated.

2.6. Determination of model drugs

2.6.1. In plasma

Plasma was deproteinized with acetonitrile after the addition of internal standard. As an internal standard, piroxicam (Sigma) was used for felbinac, flurbiprofen and ketoprofen. Flufenamic acid (Sankyo, Tokyo, Japan), *o*-anisic acid (Tokyo Chemical Industry Co.), oxprenolol (Sigma), flurbiprofen, zomepirac and tolmetin were used for diclofenac, salicylic acid, propranolol, naproxen, tolmetin and zomepirac, respectively. An aliquot of the supernatant was introduced onto the HPLC system described below.

2.6.2. In viable skin and muscle

Viable skin and muscle were excised at fixed time periods and each was placed into the centrifuging tube, where an internal standard and 1.5 ml of 0.5 N NaOH was added and the tissues were solubilized in a boiling water bath for 30 min. For felbinac, flurbiprofen, ketoprofen, naproxen, tolmetin and zomepirac, the mixtures were shaken with dichloromethane and the aqueous phase was extracted with dichloromethane after adding 3 N HCl. In the case of diclofenac and salicylic acid, hexane, diethylether and/or chloroform were used for the extraction instead of dichloromethane. For propranolol, after the solubilization of the tissues, the mixtures were back-extracted with chloroform under the acidic condition and the obtained aqueous phase was extracted with chloroform under the basic condition. The obtained residue after evaporating dichloromethane was dissolved in the mobile phase used in HPLC analysis and injected onto an HPLC system. The HPLC system consisted of a model LC-6A HPLC pump (Shimadzu, Kyoto) and a UV detector (SPD-6A; Shimadzu) set at 280, 230, 290, 254, 260, 254, 260, 330 and 330 nm for diclofenac, salicylic acid, propranolol, felbinac, flurbiprofen, ketoprofen, naproxen, tolmetin and zomepirac, respectively. Analytical column was Inertsil $5C_{18}$ (150×4.6 mm I.D., GL Sciences, Tokyo). The mobile phase was delivered at 1 ml/min and the composition was as follows: 20 mM phosphate buffer (pH 7.4)–acetonitrile (62:38, v/v for felbinac; 80:20 for flurbiprofen;

70:30 for ketoprofen; 75:25 for naproxen, tolmetin and zomepirac); methanol–acetonitrile–22 mM acetate buffer (pH 7.4) (25:25:50, v/v for diclofenac); 0.5% phosphoric acid–acetonitrile (75:25, v/v for salicylic acid); 10% acetic acid–methanol (60:40, v/v for propranolol). For quantitative calculations, a Shimadzu C-R6A data module was employed. The coefficient of variation (CV) for each standard curve ranged from 0.1 to 13.0% and the squared correlation coefficient was over 0.994 for all the model drugs examined in the present study.

2.7. Pharmacokinetic analysis with six-compartment model

Based on the compartment models composed of donor cell, viable skin, muscle, plasma, contralateral skin and contralateral muscle, as shown in Fig. 1, the linear differential equations were obtained as follows.

Donor cell

$$V_{\mathrm{d}}\cdot\frac{dC_{\mathrm{d}}}{dt} = -CL_{\mathrm{d-vs}}\cdot C_{\mathrm{d}} \tag{1}$$

Viable skin

$$V_{\mathrm{vs}}\cdot\frac{dC_{\mathrm{vs}}}{dt} = CL_{\mathrm{d-vs}}\cdot C_{\mathrm{d}} + CL_{\mathrm{m-vs}}\cdot C_{\mathrm{m}} + CL_{\mathrm{p-vs}}\cdot C_{\mathrm{p}} - (CL_{\mathrm{vs-m}} + CL_{\mathrm{vs-p}})\cdot C_{\mathrm{vs}} \tag{2}$$

Muscle

$$V_{\mathrm{m}}\cdot\frac{dC_{\mathrm{m}}}{dt} = CL_{\mathrm{vs-m}}\cdot C_{\mathrm{vs}} + CL_{\mathrm{p-m}}\cdot C_{\mathrm{p}} - (CL_{\mathrm{m-vs}} + CL_{\mathrm{m-p}} + CL_{\mathrm{m-}})\cdot C_{\mathrm{m}} \tag{3}$$

Contralateral viable skin

$$V_{\mathrm{cs}}\cdot\frac{dC_{\mathrm{cs}}}{dt} = CL_{\mathrm{m-vs}}\cdot\frac{V_{\mathrm{cm}}}{V_{\mathrm{m}}}C_{\mathrm{cm}} + CL_{\mathrm{p-vs}}\cdot\frac{V_{\mathrm{cs}}}{V_{\mathrm{vs}}}\cdot C_{\mathrm{p}} - (CL_{\mathrm{vs-m}} + CL_{\mathrm{vs-p}})\cdot\frac{V_{\mathrm{cs}}}{V_{\mathrm{vs}}}C_{\mathrm{cs}} \tag{4}$$

Contralateral muscle

$$V_{\mathrm{cm}}\cdot\frac{dC_{\mathrm{cm}}}{dt} = CL_{\mathrm{vs-m}}\cdot\frac{V_{\mathrm{cs}}}{V_{\mathrm{vs}}}\cdot C_{\mathrm{cs}} + CL_{\mathrm{p-m}}\cdot\frac{V_{\mathrm{cm}}}{V_{\mathrm{m}}}\cdot C_{\mathrm{p}} - (CL_{\mathrm{m-vs}} + CL_{\mathrm{m-p}} + CL_{\mathrm{m-}})\cdot\frac{V_{\mathrm{cm}}}{V_{\mathrm{m}}}\cdot C_{\mathrm{cm}} \tag{5}$$

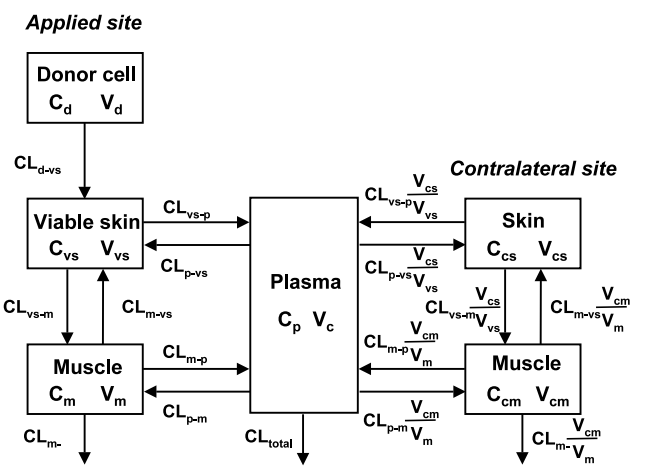

Fig. 1. Scheme of six-compartment model describing intradermal kinetics of drugs applied topically. C and V reveal the concentration and the distribution volume, respectively. Subscripts of d, vs, m, p, cs and cm reveal the donor cell, viable skin, muscle, plasma, contralateral viable skin and contralateral muscle compartments, respectively. CL indicates the clearance between tissue and tissue or plasma as represented by subscripts. All the clearances, except for CL_{total}, are calculated as unit of viable skin or muscle mass.

Plasma

$$V_p \cdot \frac{dC_p}{dt} = CL_{vs-p} \cdot \left(C_{vs} + \frac{V_{cs}}{V_{vs}} \cdot C_{cs} \right) + CL_{m-p} \cdot \left(C_m + \frac{V_{cm}}{V_m} \cdot C_{cm} \right) - \left(CL_{p-vs} + CL_{p-m} + CL_{p-vs} \cdot \frac{V_{cs}}{V_{vs}} + CL_{p-m} \cdot \frac{V_{cm}}{V_m} + CL_{total} \right) \cdot C_p \quad (6)$$

where V and C mean the distribution volume and drug concentration in each compartment, respectively. Subscripts of d, vs, m, cs, cm and p reveal the donor cell, viable skin, muscle, contralateral skin, contralateral muscle and plasma compartments, respectively. CL indicates the clearance between tissue and tissue or plasma as represented by subscripts.

Drug concentration–time curves observed were simultaneously fitted with the differential equations described above, using MULTI (RUNGE) (Yamaoka and Nakagawa, 1983), a nonlinear least-square regression program by Damping Gauss–Newton algorithm and the CL values were obtained. Mean value of each tissue weight, obtained experimentally, was used as a distribution volume of viable skin (0.26 g) or muscle (1.47 g) under the area applied topically. As CL_{total} and V_p were used the values of CL_{total} and Vd_1, the distribution volume of the central compartment, obtained in the intravenous administration study and CL_{m-vs} was assumed to be $CL_{vs-m} \cdot Kp_{s/m}$. Although the equation, $V_p = Vd_{ss} - Kp_{s/p} \cdot V_{cs} - Kp_{m/p} \cdot V_{cm}$, was used to calculate the V_p value in the previous report (Nakayama et al., 1999), the V_p values obtained according to the equation above gave a negative value for some drugs examined in the present study. Therefore, V_p was simply assumed to be Vd_1 as described above and the results for antipyrine were re-analyzed in the present study. $Kp_{s/p}$, $Kp_{s/m}$ or $Kp_{m/p}$ represents the ratio of drug concentration in the viable skin to that in plasma or muscle, or the ratio of that in muscle to that in plasma, respectively. As all the clearances, except for CL_{total}, are calculated as unit of viable skin or muscle mass under application site, the correction by distribution volume is necessary, as described above, to estimate the substantial value of clearance for contralateral tissues. In this model analysis, it was assumed that (a) contralateral viable skin and muscle mean all those except the applied site. (b) CL (per gram of tissue) between plasma and any tissue is equal without any distinction of applied or non-applied sites. The final fitting was deemed acceptable based on the regression goodness-of-fit criteria, the Akaike's information criteria (Yamaoka et al., 1978).

2.8. Statistical analysis

Results are expressed as the mean ± S.E. of more than four experiments. Statistical significance of the correlation between observed and calculated values were determined by Pearson's method, which is a method to estimate the significance for the linear correlation by calculating Pearson's correlation coefficient. The multiple linear regression analysis was performed by using EXCEL multivariate analysis program (ESUMI Co. Ltd., Tokyo).

3. Results

Ten model compounds were examined in terms of the intradermal disposition after topical application. Some of the physiochemical parameters are summarized in Table 1, showing the compounds examined ranged between 138.1 and 295.1 in molecular weight (Mw), between −0.512 and 1.259 in Log P and between 0.401 and 0.998 in the unbound fraction (fu_{vs}) in the viable skin (27% homogenate). In-vivo transdermal absorption study was performed for six model compounds and the observed data were analyzed by the six-compartment model (Fig. 2). The fitting lines obtained described the drug concentration–time profiles in each tissue very well and the lines were significantly correlated with the observed values for all six drugs. The clearance values obtained are listed in Table 2, showing that the clearances between the viable skin and the muscle are much smaller than those between the viable

Table 1
Physicochemical parameters of model compounds

Drugs	Mw[a]	pKa	Log P[b]	fu_{vs}[c]
Antipyrine	188.2	1.40	0.388	0.998
Diclofenac	295.1	4.16	1.259	0.592
Salicylic acid	138.1	2.97	−0.512	0.870
Propranolol	259.3	9.23	1.195	0.550
Ketoprofen	254.3	3.90	−0.275	0.701
Felbinac	212.2	–	0.293	0.667
Flurbiprofen	244.3	3.78	1.068	0.401
Naproxen	230.2	4.90	0.276	0.628
Tolmetin	257.3	3.60	−0.341	0.726
Zomepirac	291.7	–	0.062	0.631

[a] Molecular weight.
[b] Logarithm of the partition coefficient between *n*-octanol/pH 7.4 isotonic phosphate buffer at 37 °C.
[c] Unbound fraction in 27% viable skin homogenate.

skin and the plasma and between the muscle and the plasma.

Using the parameters obtained above, we calculated the drug concentration in muscle-time profiles derived from the direct penetration or from the distribution via the systemic circulation (Fig. 3). The calculated profiles show that the direct penetration is predominant for all six model drugs, especially at the early period of time after starting the absorption study. The relative contribution of the direct penetration or the distribution from the systemic circulation (Table 3), which was calculated based on their AUC values of the calculated curves of Fig. 3, indicates that there is a variability in the contribution of the direct penetration to the drug disposition into the muscular layer after topical application. The muscular disposition of diclofenac was almost attributed to the direct penetration (90.8%), although $>50\%$ of felbinac was distributed there via the systemic circulation.

Then, CL_{vs-m}, the clearance for the direct penetration into the muscular layer, was investigated in terms of the relationship with Mw, Log P or fu_{vs}. Only fu_{vs} showed the statistically significant and positive correlation with CL_{vs-m} (Fig. 4(A)), although the relation between CL_{vs-m} and Mw indicated the tendency of the negative correlation (data not shown). The results strongly suggest that fu_{vs} could be one of the important factors determining the penetration into the muscular layer. Fig. 4(B) shows the relationship between CL_{vs-p} and fu_{vs}, indicating that CL_{vs-p} also tends to increase with the increase in fu_{vs}. However, as shown in Fig. 4(C), the ratio of CL_{vs-m} to CL_{vs-p} significantly increases as fu_{vs} value becomes larger. This result clearly means that the increase in fu_{vs} in the viable skin contributes more to the enhancement of the direct penetration than that of the systemic absorption.

On the other hand, k_{direct} values obtained by in vitro penetration study show the good correlation with CL_{vs-m} values obtained by in vivo study (Fig. 5). Therefore, the in vitro penetration study was performed for the other three drugs, naproxen, tolmetin and zomepirac and the multiple linear regression analysis of k_{direct} was performed for ten model drugs in terms of fu_{vs}, Log P and Mw. The value of k_{direct} was significantly explained by the three factors (Fig. 6(A) and Table 4) and fu_{vs} showed significantly the largest contribution to the direct penetration into the muscular layer. Furthermore, concerning with k_{direct}/fu_{vs}, the multiple regression analysis was carried out in terms of Log P and Mw (Fig. 6(B) and Table 4). Although the result was not statistically significant, Mw was suggested to be a secondary important factor, acting to decrease the direct penetration of drugs.

4. Discussion

For the transdermal delivery system aiming at local therapy, such as a treatment for muscle aches and relief of joint pain, the enhancement of local availability must be the aim, as well as overcoming the stratum corneum barrier. The direct penetration of drugs from the viable skin must be an important process for the improvement of AUC in the muscular layer after topical application. It is still a controversial situation whether the drug penetrates into deeper tissues after topical application or is removed by the dermal blood supply to the skin (Cross et al., 1997). In the present study, seven model drugs, including antipyrine (Nakayama et al., 1999), were examined based on the six-compartment model (Fig. 1) in terms of the intradermal kinet-

Table 2
Clearances describing intradermal kinetics of drugs in in vivo transdermal absorption study

Drugs	CL_{total} (ml/h)	CL_{d-vs} (ml/h)	CL_{vs-m} (ml/h)	CL_{m-vs}[b] (ml/h)	CL_{vs-p} (ml/h)	CL_{p-vs} (ml/h)	CL_{m-p} (ml/h)	CL_{p-m} (ml/h)	CL_{m-} (ml/h)
Antipyrine[a]	64.68 (2.72)	3.43×10^{-1} (0.16×10^{-1})	3.81×10^{-1} (0.10×10^{-1})	3.27×10^{-1}	2.85 (0.00)	3.80 (0.29)	0.87 (0.11)	0.85 (0.34)	1.90×10^{-3} (7.61×10)
Diclofenac	253 (23)	4.99×10^{-1} (0.14×10^{-1})	0.08×10^{-1} (0.00×10^{-1})	0.23×10^{-1}	1.38 (0.00)	0.75[c]	2.26 (0.39)	0.39 (0.01)	2.17×10^{-6} (2.95×10^{3})
Salicylic acid	6.63 (0.76)	4.07×10^{-1} (0.16×10^{-1})	0.85×10^{-1} (0.04×10^{-1})	2.25×10^{-1}	1.92 (0.01)	0.59[c]	1.12 (0.17)	0.18 (0.01)	1.05×10^{-4} (6.91×10^{2})
Propranolol	680 (106)	8.78×10^{-1} (0.32×10^{-1})	0.31×10^{-1} (0.01×10^{-1})	0.45×10^{-1}	2.40 (0.01)	3.79 (0.05)	1.10 (2.95)	1.35 (0.05)	4.36×10^{-4} (2.04×10^{-1})
Ketoprofen	30.75 (0.51)	2.05×10^{-1} (0.19×10^{-1})	1.33×10^{-1} (0.69×10^{-1})	3.20×10^{-1}	1.24 (0.01)	0.56 (0.03)	6.53 (4.08)	1.49 (0.96)	1.96×10^{-3} (3.82×10)
Felbinac	7.67 (1.32)	4.46×10^{-1} (0.41×10^{-1})	0.43×10^{-1} (0.00×10^{-1})	0.17×10^{-1}	2.24 (0.00)	0.44 (0.00)	2.45 (0.03)	0.37 (0.00)	7.75×10^{-5} (1.81×10)
Flurbiprofen	6.60 (0.40)	4.23×10^{-1} (0.28×10^{-1})	0.32×10^{-1} (0.00×10^{-1})	0.86×10^{-1}	$1.55\times(0.00)$	0.40 (0.00)	0.83 (0.04)	0.15 (0.00)	9.93×10^{-3} (3.07×10^{-2})

Clearances except for CL_{total} were obtained by the fitting study based on the six-compartment models and shown with S.D. values in parentheses. The values of CL_{total} were obtained by the intravenous administration studies. The meaning of each clearance is as follows: CL_{total}, total body clearance; CL_{d-vs}, CL from donor cell to viable skin; CL_{vs-m}, CL from viable skin to muscle; CL_{m-vs}, muscle to viable skin; CL_{vs-p}, viable skin to plasma; CL_{p-vs}, plasma to viable skin; CL_{vs-m}, viable skin to muscle; CL_{m-p}, muscle to plasma; CL_{p-m}, plasma to muscle; CL_{m-}, muscle to deeper tissue.

[a] Recalculated based on the results of Nakayama et al. (1999).

[b] Calculated based on the equation, $CL_{m-vs} = CL_{vs-m} \cdot Kp_{s/m}$.

[c] Calculated based on the equation, $CL_{p-vs} = CL_{vs-p} \cdot Kp_{s/p}$.

ics. The fitting lines obtained described the concentration in each tissue-time profiles for all the drugs examined very well (Fig. 2), showing that the model is useful for the analysis and description of the intradermal disposition of drugs after topical application. The simulation study using the parameters (Table 2) gave us some answer for the controversial issue of whether topically applied drugs substantially penetrate into the muscle layer directly from the viable skin or not. Since our model includes the contralateral skin and muscle, the contribution of the absorption from the systemic circulation to the muscular disposition can be evaluated precisely. Drugs with a variety of physicochemical characteristics were shown to be able to penetrate directly into the muscular layer (Fig. 3), supporting the report by Yanagimoto et al. (1998). They showed that most drugs in the muscle was via direct penetration from the skin. Except for felbinac, over 50% of drugs distributed into the muscle were attributed to direct penetration from the viable skin for 10 h after application (Table 3). In our simulation study, however, the disposition via the systemic circulation was also recognized at the same time. Singh and Roberts (1993) suggested that the direct penetration of salicylic acid only occurred during the first 2 h of application and that systemic circulation of salicylic acid through the tissues dominated the observed tissue concentrations at longer times. As shown in Fig. 3, even in the case of felbinac, the direct penetration was predominant during the early periods and the systemic circulation came to supply more drugs

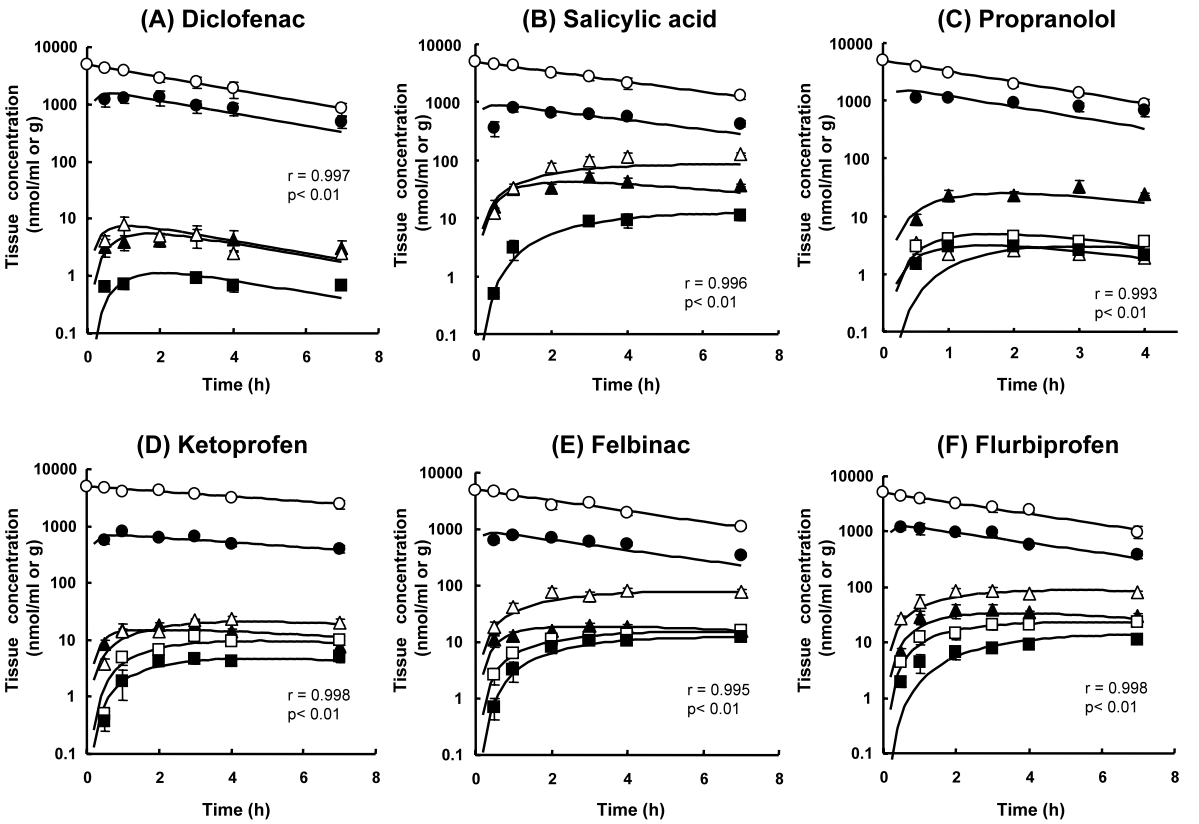

Fig. 2. Intradermal kinetics of six model drugs after topical application on the stripped skin in vivo. Results are expressed as the mean with a vertical bar showing the S.E. of more than four experiments. (A) Diclofenac; (B) salicylic acid; (C) propranolol; (D) ketoprofen; (E) felbinac; (F) flurbiprofen. Solid lines represent the obtained fitting curves. Keys: ○, donor cell; ●, viable skin; △, plasma; ▲, muscle; □, contralateral viable skin; ■, contralateral muscle.

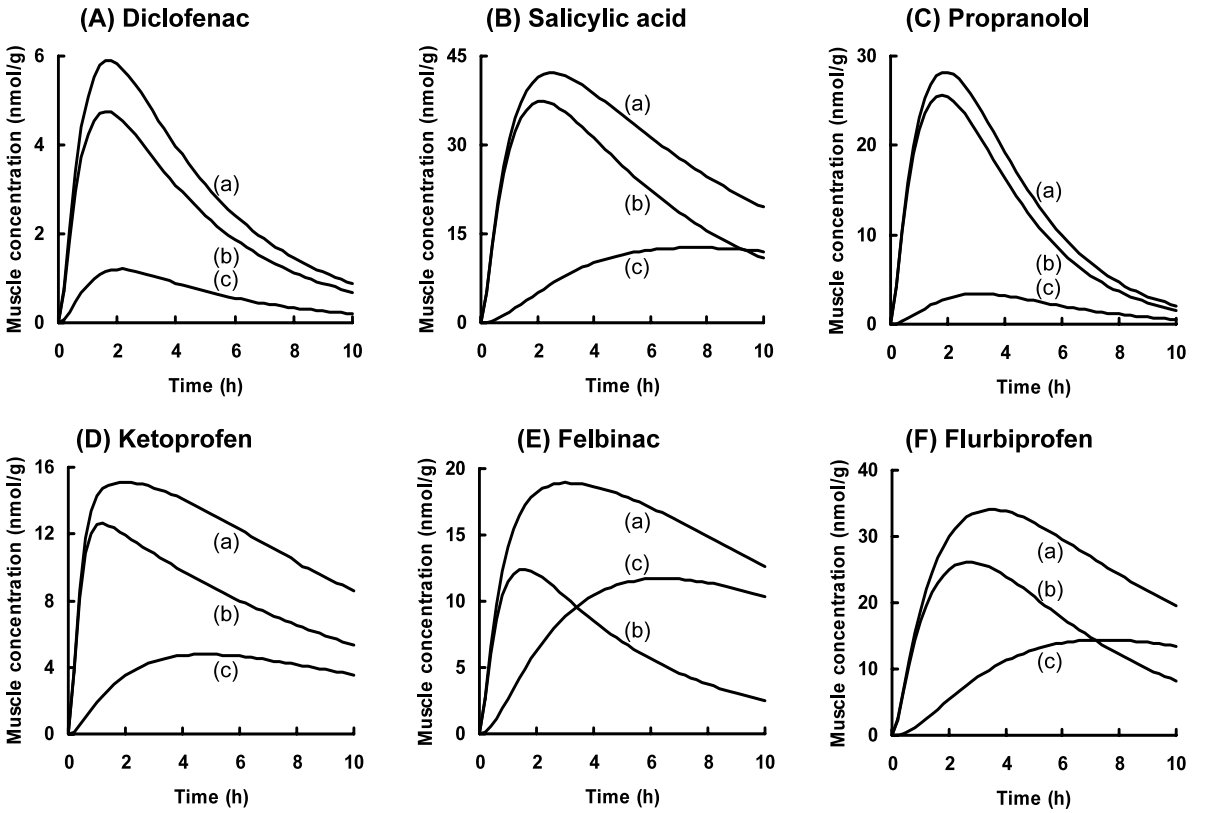

Fig. 3. Evaluation of direct penetration and transport from systemic circulation to muscle layer below application site. Simulation curves were described using the parameters obtained by six-compartment model analysis. (A) Diclofenac; (B) salicylic acid; (C) propranolol; (D) ketoprofen; (E) felbinac; (F) flurbiprofen. Keys: (a–c) indicate the total concentration, the concentration due to direct penetration and the concentration due to systemic circulation, respectively.

than the direct penetration did for felbinac, flurbiprofen and salicylic acid after 4, 7 and 10 h of topical application, respectively. Ketoprofen in the muscle would also be derived from the systemic circulation more than the direct penetration during the later periods than 10 h. Although the tendency might also be dependent on the weight function, i.e. the pharmacokinetics of drugs after entering the systemic circulation, the present results suggest that drug supply via direct penetration was predominant during the early periods and that the contribution of the systemic circulation was becoming larger over longer times.

CL_{vs-m}, the clearance for the direct penetration to the muscular layer, was analyzed in terms of the relationship with Mw, Log P or fu_{vs} and the result suggested that fu_{vs} plays the most important role in the direct penetration among the three factors (Fig. 4(A)). As k_{direct} obtained in the in vitro study was found to reflect CL_{vs-m} (Fig. 5), the multiple linear regression analysis of k_{direct} was performed for ten model drugs concerned with Mw, Log P and fu_{vs} (Fig. 6(A) and Table 4). The results strongly suggested that fu_{vs} should be one of the important factors for penetration from the viable skin. The same analysis of k_{direct}/fu_{vs} in terms of Log P and Mw suggested that Mw might correlate with k_{direct}/fu_{vs} (Fig. 6(B)), although it was not statistically significant. As the molecular size of the compound was suggested to be one of the critical factors for diffusing (Nara et al., 1992; Singh and Roberts, 1996), Mw might be another important factor. Although it is well known that the high lipophilicity is needed for overcoming the

Table 3
Relative contribution of direct penetration and distribution from systemic circulation to drugs in muscle layer after topical application

Drugs	Direct penetration (%)	Distribution from systemic circulation (%)
Antipyrine[a]	90.8	9.2
Diclofenac	79.0	21.0
Salicylic acid	72.0	28.0
Propranolol	86.1	13.9
Ketoprofen	69.4	30.6
Felbinac	43.3	56.7
Flurbiprofen	62.5	37.5

The relative contribution of the two pathways was calculated based on the AUC values from 0 to 10 h in Fig. 3.
[a] Recalculated based on the results of Nakayama et al. (1999).

stratum corneum barrier, it was suggested that this is not the case for the muscular penetration from the viable skin (Singh and Roberts, 1994; Nakayama et al., 1999). The present analysis also shows that the lipophilicity of drugs might not be so important (Table 4).

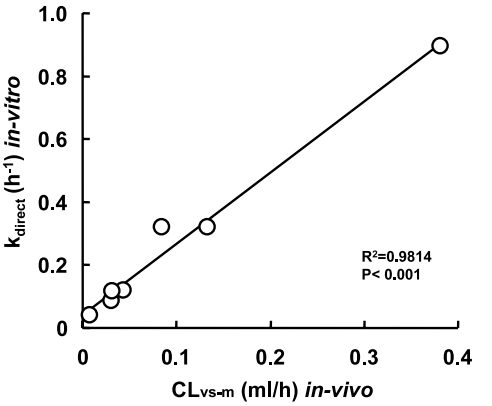

Fig. 5. Relationship between CL_{vs-m} (in vivo) and k_{direct} (in vitro). CL_{vs-m} and k_{direct} mean the clearance from viable skin to muscle obtained by in vivo study and the transport rate constant from viable skin to muscle obtained by in vitro study, respectively. The solid line was obtained by linear regression method.

In the present study, k_{direct} obtained by in vitro penetration study has been found to be significantly correlated with CL_{vs-m} obtained by in vivo study (Fig. 5). The reasons for the correlation between in vitro and in vivo parameters might be

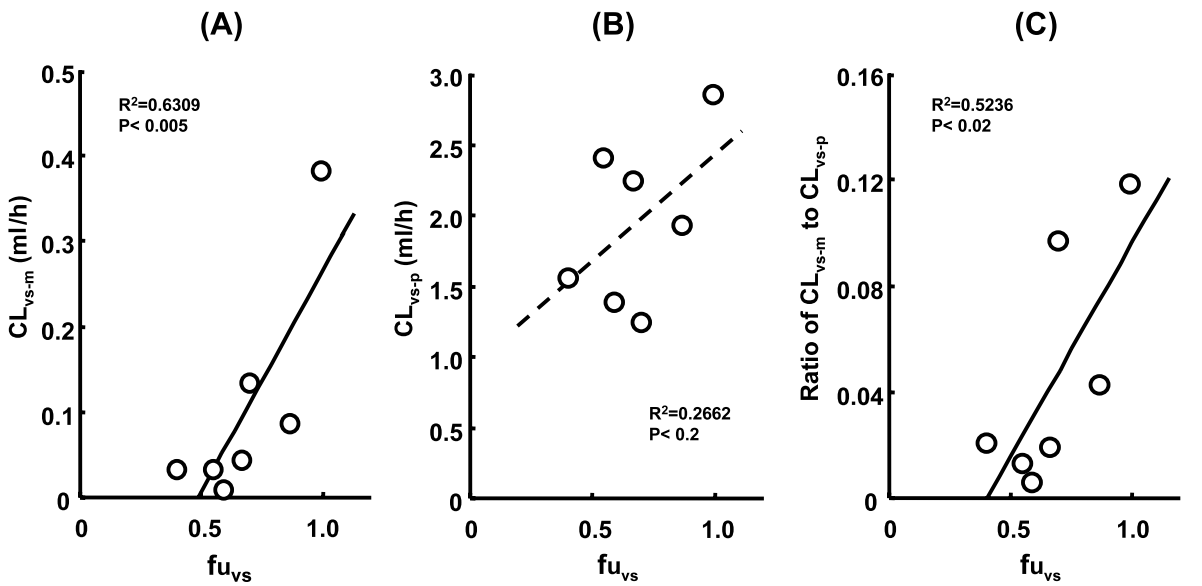

Fig. 4. Correlation of CL_{vs-m} with unbound fraction in viable skin. (A) CL_{vs-m} and fu_{vs}; (B) CL_{vs-p} and fu_{vs}; (C) ratio of CL_{vs-m} to CL_{vs-p}. CL_{vs-m} and CL_{vs-p} mean the clearance from the viable skin to the muscle and the one from the viable skin to the plasma, respectively. The solid line, statistically significant and the dotted line, not significant, were obtained by the linear regression method.

as follows: (i) drug concentration gradient between viable skin and muscular layer might be kept similar to that of the in vivo study; (ii) CL_{vs-m} can be determined as a parameter to describe the direct penetration precisely, independent of the elimination to the plasma.

Roberts and Cross (1999) suggested that the intradermal kinetics of drugs might be a function of the binding in tissue, the binding in plasma and the local blood flow. They also suggested that the balance between the unbound fraction in plasma and the unbound fraction in tissues is important for the retention of drugs in the tissues. In the present study, we have focused on the binding in the viable skin and have been able to present its importance. Drugs in the viable skin might be bound to the cytosolic components, as reported by Yagi et al. (1998). The binding in plasma might be a factor influencing the direct penetration of drugs and we have already been conducting experiments to estimate the binding in plasma and in muscle. Furthermore, the local blood flow might also regulate the muscular disposition of drugs, as epinephrine was reported to enhance the direct penetration of flurbiprofen probably because of the decrease in the blood flow (Sugibayashi et al., 1999). Our preliminary study using phenylephrine, an adrenergic α_1-agonist, also showed that the direct penetration of antipyrine was enhanced by the treatment with phenylephrine (data not shown), although further study for estimating the local blood flow rate is needed.

As shown in Fig. 4(B), CL_{vs-p} tends to be correlated with fu_{vs} as well. This tendency might be reasonable considering that a free molecule would be more diffusive than a molecule bound to protein and/or lipid, although the relationship between CL_{vs-p} and fu_{vs} was not statistically significant. Moreover, Fig. 4(C) clearly shows that the increase in fu_{vs} enlarged more CL_{vs-m} than CL_{vs-p} significantly, meaning the larger amount of unbound molecules in the viable skin could enhance the direct penetration to the muscular layer more than to the systemic absorption. The reason why the increase in the unbound fraction in the viable skin is more favorable for the direct penetration than for the systemic absorption remains unknown, but the balance in the unbound fraction among the viable skin, the muscular layer and the plasma might be a critical factor. As a further study, the pharmacokinetic analysis will be performed using the six-compartment model including the binding in the viable skin, the muscular layer and the plasma.

In conclusion, based on the six-compartment model including the contralateral tissues, drugs were suggested to be able to penetrate into the muscular layer substantially after topical application. Furthermore, the unbound fraction in the

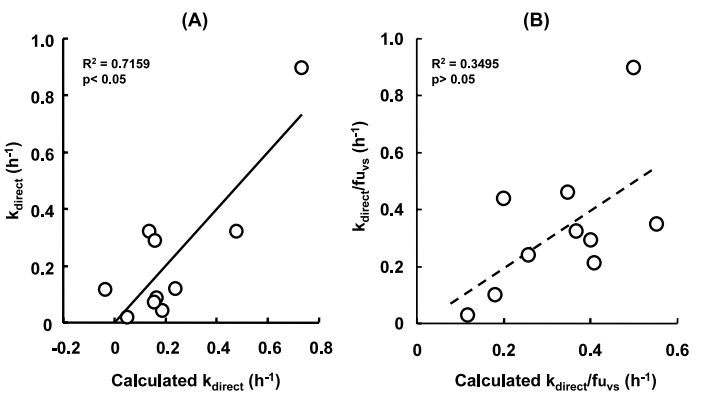

Fig. 6. Multiregression analysis of factors influencing intradermal kinetics of drugs applied topically. (A) Relationship between k_{direct} and calculated k_{direct} based on multiregression analysis. (B) Relationship between k_{direct}/fu_{vs} and calculated k_{direct}/fu_{vs} based on multiregression analysis. The solid line, statistically significant, and the dotted line, not significant, were obtained by the linear regression method.

Table 4
Contribution of possible factors determining intradermal kinetics of drugs by multiple liner regression analysis

Kinetic parameters	Factors	SPRC	S.E.	Contribution	P
k_{direct}	Mw	−0.2369	0.28263	0.1588	0.4339
	Log P	0.4007	0.27078	0.2686	0.1895
	fu_{vs}	0.8541	0.31431	0.5726	0.0348
	Intercept	-1.38×10^{-6}	0.20643	–	–
k_{direct}/fu_{vs}	Mw	−0.6555	0.33922	0.6605	0.0946
	Log P	0.3369	0.33922	0.3395	0.3537
	Intercept	-1.06×10^{-7}	0.28921	–	–

The transport rate constant from the viable skin to the muscle, k_{direct}, was obtained by the in vitro study. Mw, Log P, fu_{vs} and SPRC mean the molecular weight, the logarithm of partition coefficient, the unbound fraction in the viable skin homogenate (27%) and the standard partial regression coefficient, respectively. Contribution and P represent the contribution ratio of each factor to k_{direct} or k_{direct}/fu_{vs} and its level of significance, respectively.

viable skin is possibly one of the most important factors to regulate the direct penetration of drugs from the viable skin to the muscular layer.

References

Cross, S.E., Anderson, C., Thompson, M.J., Roberts, M.S., 1997. Is there tissue penetration after application of topical salicylate formulations? Lancet 350, 363.

Dawson, M., McGee, C.M., Vine, J.H., Nash, P., Watson, T.R., Brooks, P.M., 1988. The disposition of biphenylacetic acid following topical application. Eur. J. Clin. Pharmacol. 33, 639–642.

Hadgraft, J., Walters, K.A., Wotton, P.K., 1985. Facilitated transport of sodium salicylate across an artificial lipid membrane by Azone. J. Pharm. Pharmacol. 37, 725–727.

Hashida, M., Mukai, E., Kimura, T., Sezaki, H., 1985. Enhanced delivery of mitomycin C prodrugs through the skin. J. Pharm. Pharmacol. 37, 542–544.

Marty, J.P., Guy, R.H., Maibach, H.I.I., 1989. Percutaneous penetration as a method of delivery to muscle and other tissues. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery, second ed. Marcel Dekker, New York, pp. 511–529.

M-Riviere, N.A., Inman, A.O., Riviere, J.E., McNeill, S.C., Francoeur, M.L., 1993. Topical penetration of piroxicam is dependent on the distribution of the local cutaneous vasculature. Pharm. Res. 10, 1326–1331.

Nakamura, Y., Takayama, K., Higashiyama, K., Suzuki, T., Nagai, T., 1996. Promoting effect of *o*-ethylmenthol on the percutaneous absorption of ketoprofen. Int. J. Pharm. 145, 29–36.

Nakayama, K., Matsuura, H., Asai, M., Ogawara, K., Higaki, K., Kimura, T., 1999. Estimation of intradermal disposition kinetics of drugs: I. Analysis by compartment model with contralateral tissues. Pharm. Res. 16, 302–308.

Nara, E., Masegi, M., Hatono, T., Hashida, M., 1992. Pharmacokinetic analysis of drug absorption from muscle based on a physiological diffusion model: effect of molecular size on absorption. Pharm. Res. 9, 161–168.

Palagiano, F., Arenare, L., Barbato, F., Rotonda, M.I.L., Quaglia, F., Bonina, F.P., Montenegro, L., de Caprariis, P., 1997. In vitro and in vivo evaluation of terpenoid esters of indomethacin as dermal prodrugs. Int. J. Pharm. 149, 171–182.

Radermacher, J., Jentsch, D., Scholl, M.A., Lustinetz, T., Frolich, J.C., 1991. Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br. J. Clin. Pharmacol. 31, 401–418.

Riviere, J.E., Heit, M.C., 1997. Electrically-assisted transdermal drug delivery. Pharm. Res. 14, 687–697.

Roberts, M.S., Cross, S.E., 1999. A physiological pharmacokinetic model for solute disposition in tissues below a topical application site. Pharm. Res. 16, 1392–1398.

Singh, P., Roberts, M.S., 1993. Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. J. Pharmacokinet. Biopharm. 21, 337–373.

Singh, P., Roberts, M.S., 1994. Dermal and underlying tissue pharmacokinetics of lidocaine after topical application. J. Pharm. Sci. 83, 774–782.

Singh, P., Roberts, M.S., 1996. Local deep tissue penetration of compounds after dermal application: structure–tissue penetration relationships. J. Pharmacol. Exp. Ther. 279, 908–917.

Singh, P., Roberts, M.S., Maibach, H.I., 1995. Modelling of plasma levels of drugs following transdermal iontophoresis. J. Control. Rel. 33, 293–298.

Sugibayashi, K., Yanagimoto, G., Hayashi, T., Seki, T., Juni, K., Morimoto, Y., 1999. Analysis of skin disposition of flurbiprofen after topical application in hairless rats. J. Control. Res. 62, 193–200.

Williams, C., Barry, B.W., 1992. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Sys. 9, 305–353.

Yagi, S., Nakayama, K., Kurosaki, Y., Higaki, K., Kimura, T., 1998. Factors determining drug residence in skin during transdermal absorption: studies on β-blocking agents. Biol. Pharm. Bull. 21, 1195–1201.

Yamaoka, K., Nakagawa, T., 1983. A nonlinear least squares program based on differential equations, multi (runge), for microcomputers. J. Pharmacobio.-Dyn. 6, 595–606.

Yamaoka, K., Nakagawa, T., Uno, T., 1978. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J. Pharmacokinet. Biopharm. 6, 165–175.

Yanagimoto, G., Hayashi, T., Seki, T., Juni, K., Sugibayashi, K., Morimoto, Y., 1998. A new in-situ experimental method to evaluate the cutaneous disposition of flurbiprofen after topical application in hairless rats. Pharm. Pharmacol. Commun. 4, 261–265.